IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Johnson, et al.	Art Group Unit: TBA
Filed:	September 18, 2003	Examiner: TBA
Serial No.:	TBA	
For:	Purification of Taxanes and Taxane Mixtures Using Polyethyleneimine-Bonded Resin	

AFFIDAVIT OF JAMES H. JOHNSON, Ph.D.

I, James H. Johnson, Ph.D, residing at 6 Central St., Merrimac, MA 01860, hereby declare:

1. I am a named co-inventor of the above-captioned application.

2. I am making this affidavit pursuant to my duty to disclose information that may be material to the patentability of the invention claimed in the present application.

3. I am currently Vice President of Research & Development at Natural Pharmaceuticals, Inc. ("NPI"). NPI is the assignee of the present application.

4. I received a Ph.D from the University of Florida, Dept. of Medicinal Chemistry, 1998.

5. On information and belief, prior to March 17, 2003 (at least one year prior to the earliest priority date of the present application), the following events occurred.

1. Purification of Biomass Extract Using PEI Resin

6. During November of 2000, NPI employed a process using a Polyethyleneimine-bonded silica chromatographic resin ("PEI resin") to purify a biomass extract comprising a mixture of taxanes. The extract (i.e., starting material) containing the taxanes was purchased

from a third-party supplier. To the best of my knowledge, the biomass extract had already gone through various purification procedures by the supplier, including biomass extraction, precipitation, and two chromatography procedures. The biomass source from which the extract was obtained was *Taxus brevifolia* (Pacific yew). On information and belief, the extract comprised about 25 %-40 % primary taxanes by weight (including taxol A, B, C, D, E, F, or G) of the total solids. (See Attachment 1 for structures of primary taxanes).

7. NPI dissolved the *Taxus brevifolia* extract in acetone containing 0.4-0.6% acetic acid. NPI then purified the extract by passing it through columns packed with PEI resin in a simulated moving bed (SMB) fashion. Specifically, the extract was loaded onto J.T. Baker PEI resin (40 micron particle size, 275 angstrom pore size, J. T. Baker Item # 7264).

8. The taxanes were eluted from the PEI resin with an eluant. The eluant was acetone with 0.4-0.6% acetic acid. The taxanes were recovered in one or more fractions of eluate. The taxanes were then crystallized. The crystallized material was then subject to a semi-synthetic process to produce a crude mixture of taxol A (Paclitaxel), described below.

2. Separation And Isolation of Taxol A (Paclitaxel) From Semi-Synthetic Mixture Using DEAM Resin

9. The material produced above was subjected to a semi-synthetic process to form a crude semi-synthetic mixture of taxol A (Paclitaxel). The crude taxol A mixture was prepared via the primary amine conversion process described in U.S. Serial Appl. No. PCT/US03/10557 entitled "Conversion of Taxane Molecules." Generally, this process included the steps of benzoylating the C-2' hydroxyl group of the taxane molecule; reductively deoxygenating the taxane molecule to form an imine compound; hydrolyzing the imine compound with acid; and treating the taxane amine with base to effect acyl migration of the C-2'-O-acyl group to the C-3' primary amine.

10. To the best of my knowledge, the resulting crude semi-synthetic mixture had high amounts of C-2' benzoyl primary taxanes. Typically, in the samples that were tested, the amount of C-2' benzoyl primary taxanes was greater than 8 % by weight C-2' benzoates, including taxol A, B, C, D, F, or G. Also, to the best of my knowledge, it had greater than 0.5 % by weight taxol B, C, D, E, F, or G.

11. After work-up of the acyl migration reaction, the desired taxane molecule (taxol A) was isolated by passing the mixture through a column with Diethylaminomethyl (DEAM) Bonded Silica Gel having 20 micron particle size, and 120 Angstrom average pore size.

12. Before passing the crude taxol A mixture through the DEAM packed column, the mixture was dissolved using a solvent comprising ethyl acetate with 1-10% (vol.) tetrahydrofuran. The solution was stirred with mild heating. The solution was then vacuum filtered to remove any small fibers or particulates before injection. After filtration, the solution was diluted using EtOAc solution. The entire volume of the solution was then injected on the DEAM column and the taxol A was eluted using a mobile phase consisting of ethyl acetate with 0.5% (vol.) acetic acid. Fractions comprising taxol A were collected.

13. After most (>90%) of the taxol A had eluted from the column a linear gradient to 40-50% (vol.) methanol was utilized as a wash solvent mobile phase. This wash step allowed for faster elution of the 10-deacetyltaxol A compound that elutes after taxol A.

14. The purity of the crude material going onto the column was greater than 60 wt. % taxol A. After column purification, the purity of the combined fractions was greater than 90 wt. % taxol A.

15. The purified taxol A (Paclitaxel) was sold prior to March 17, 2002.

16. I hereby declare that all statements made herein are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Dated: September ___, 2003

By: _____
James H. Johnson, Ph.D.

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